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Neuronal Nicotinic Acetylcholine Receptor Expression and Function on Nonneuronal Cells

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ABSTRACT

Of the thousands of proven carcinogens and toxic agents contained within a cigarette, nicotine, while being the addictive agent, is often viewed as the least harmful of these compounds. Nicotine is a lipophilic molecule whose effects on neuronal nicotinic acetylcholine receptors (nAChR) have been primarily focused on its physiologic impact within the confines of the brain and peripheral nervous system. However, recently, many studies have found neuronal nAChRs to be expressed on many different nonneuronal cell types throughout the body, where increasing evidence suggests they have important roles in determining the consequences of nicotine use on multiple organs systems and diseases as diverse as ulcerative colitis, chronic pulmonary obstructive disease, and diabetes, as well as the neurologic disorders of Parkinson's and Alzheimer's disease. This review highlights current evidence for the expression of peripheral nAChRs in cells other than neurons and how they participate in fundamental processes, such as inflammation. Understanding these processes may offer novel therapeutic strategies to approach inflammatory diseases, as well as precautions in the design of interventional drugs.

KEYWORDS: nicotine, inflammation, nicotinic receptors, nonneuronal

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChR) are ligand-gated ion channels whose genetics and functional properties have been studied largely for their role in modulating neurotransmission. This receptor system has also been recognized as a participant in the progression of severe pathologies of the brain. For example, the high affinity nicotine receptors are among the first (if not the first) neurotransmitter system whose expression is diminished in Alzheimer's disease.[1] Subsequent studies[2-4] have suggested that chronic nicotine administration might in fact play a beneficial role in slowing the progression of this disease. While this finding is controversial, there is now ample evidence supporting a therapeutic benefit from nicotine in Parkinson's[5] disease, and as a neuroprotectant to toxic insults such as excitotoxins[6-10] or Beta-amyloid derived peptides.[10-12] Understanding the mechanistic basis for these and other similarly interesting findings,[13] including a cognitive benefit from nicotine,[2] would be of obvious importance. The name "neuronal" was based principally on the tissue source of the DNA libraries from which these receptors were first cloned, the brain,[14] but growing evidence indicates that cells other than neurons throughout the body express these receptors[15,16] including lymphocytes, macrophages, dendritic cells, adipocytes, keratinocytes, endothelial cells, and epithelial cells of the intestine and lung. This extended expression of nAChRs is of importance because, in addition to their regulation by endogenous agonists such as acetylcholine, choline, and the exogenous compound nicotine, their impact upon peripheral processes can be quite diverse as exemplified by their ability to in some cases enhance (Crohn's disease) disease or in other cases diminish (ulcerative colitis) progression.[17-20] These apparent contradictions in the effects of nicotine are not uncommon and understanding this complex biology will in turn optimize therapeutic benefit to ensure that neuroprotective therapy for one disease does not promote immune dysfunction and the survival of unwanted cells in other tissues.

Acetylcholine Receptors

Acetylcholine receptors (see Lindstrom[21] and Hogg et al[22]) consist of 2 major subtypes, the muscarinic-activated metabotropic receptors (second messenger coupled) and the fast-ionotropic cationic nicotine-activated channel receptors, both of which are activated by the endogenous neurotransmitter, acetylcholine. Receptors of the nicotinic subclass can be distinguished further as "muscle" or "neuronal." While the muscle and neuronal nicotinic receptors exhibit similar

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sensitivity to gating by acetylcholine, the muscle receptor is much less sensitive to nicotine. Hence, at physiological concentrations, the majority of nicotine's effects are through neuronal nicotinic acetylcholine receptors (nAChR), and, in fact, when nicotine levels are sufficiently high to act upon the muscle receptor (as might occur when smokers concurrently use the transdermal nicotine patch[23]), difficulties in breathing and muscle spasms that can result in death may occur.

The mammalian nAChR family (for review see Lindstrom[21] and Hogg et al[22] and references therein) is composed of multiple members (subunits) including 7 subunits that harbor the principal components of the ligand binding site (α2, α3, α4, α6, α7, α9, and α10) and 4 structural subunits (α5, β2, β3, and β4) that impart unique functional and pharmacological properties to the receptors. In general, nAChRs fall into 3 major subgroups, the high-affinity nicotine binding receptors harboring nAChRα4, α-bungarotoxin binding proteins composed of nAChRα7, and the receptors of the autonomic nervous system composed of nAChRα3/β4 subunits. A particularly interesting aspect of nAChRs is that despite their being fast-excitatory ion channels, they may be localized in many parts of the cell including aggregates in the cell body (somal), presynaptic terminals (where they contribute to modulation of neurotransmitter release), and in or adjacent to the postsynaptic density. Because of these diverse locations, their participation in neurotransmission can be somewhat indirect as when they affect the amount of neurotransmitter released. This modulatory role directly contributes to the establishment and maintenance of tone between the excitatory and inhibitory systems.[24-31] Further, the relatively high calcium permeability of these receptors (especially nAChRα7) appears to contribute to regulating second messenger signaling pathways such as the PI3-kinase/AKT pathway,[12,32] activation of transcriptional systems such as CREB,[33] and certain proteolytic processes.[34,35] Consequently, the placement of relatively small numbers of nAChRs at key regulatory sites can lead to multiple outcomes in terms of normal cell performance and susceptibility to exogenous challenges or participation in processes ranging from neurodegeneration to inflammation. Therefore, dysregulation or modification of the function of this system may be expected to be manifested more in modifying biological or metabolic "set-points" rather than having the often dramatic on-off effects seen with other neurotransmitter receptor systems.

Despite the importance of subunit composition to nAChR expression, function, and pharmacology, the rules governing subunit assembly of nAChRs in the brain into the mature pentameric receptor are not yet known, but it is well established that nAChRs of different subunit composition exhibit very different pharmacological and functional properties. For example, nAChRs composed of α7 subunits (which bind α-bungarotoxin) exhibit almost a 10:1, Ca^{+2}:Na^{+} permeability ratio, which exceeds that of the glutamate N-methyl-D-aspartate (NMDA) receptor and the ~4:1 ratio of most other nAChRs.[21,22] This finding suggests that the activation of nAChRα7-type receptors, which also accumulate extrasynaptically, including in rafts,[36] impact upon free intracellular calcium and calcium-dependent mechanisms in a manner quite distinct from other ligand-activated ion channels as well as other nAChRs. In contrast, the majority of other nAChRs are composed of various combinations of α and β subunits. Prominent among these are receptors composed of at least nAChRα4 and β2 subunits that form the high-affinity [^{3}H]nicotine binding receptor. A curiosity of nicotine's effect on this receptor is that when ligand is in excess and present chronically, as in a smoker, the number of binding sites actually increases in a process termed "up-regulation." While the mechanism underlying this process is controversial[21,22,37]; what is clear is that not all nAChR subtypes undergo upregulation,[38] and it is imminently associated with many of the characteristics of nAChR function such as those leading to addiction.[39]

Nicotine—Agonist or Antagonist?

Although nicotine is most often considered to be an agonist or activator of nAChRs, it has several effects on receptor function that complicate this assignment and require attention in any experimental design that employs this compound. Unlike the normal ligand such as acetylcholine or choline, which are either rapidly degraded or removed from the receptor vicinity, nicotine is not readily degraded or removed. Further, because it is lipophilic, it accumulates in certain tissues well beyond the concentration suggested by measuring the serum.[40] For example, concentrations of nicotine in the brain, owing to the drug's high hydrophobicity, can reach as high as 10 μM even though its serum concentration rarely exceeds high nanomolar and is more often in the low nanogram per milliliter range. Just as nicotine partitions and concentrates in the brain due to this lipophilic nature, other tissues also have elevated levels of nicotine compared with blood. It has been reported, using an animal model of nicotine infusion,[41] that the tissue-to-blood ratios of nicotine in different parts of the body are the following: brain 3.0, heart 3.7, muscle 2.0, adipose tissue 0.5, kidney 21.6, liver 3.7, lung 2.0, and gastrointestinal tissue 3.5. Therefore, several tissues can reach nicotine levels that approximate those achieved in the brain. As such, the duration and persistence of nicotine administration over time becomes an important pharmacological variable in the use and interpretation of this drug's actions.

These issues of concentration and duration of nicotine exposure lead to the important role that receptor desensitization, or closing of the receptor in the sustained presence of

agonists, plays in understanding nicotine biology and also introduces the concept that nicotine can actually be a powerful antagonist of receptor function. Under normal conditions, desensitization is obviously a fortunate feature of this system since leaving a pore in the membrane open for prolonged periods of time would lead to death. Also, under normal conditions, desensitization is likely to be relatively brief and the receptor can reset to again be opened when a burst of agonist occurs. In contrast to this physiological state, the accumulation of nicotine and unceasing receptor binding results in sustained desensitization and in some cases can actually lead to complete receptor inactivation.[22] When lower concentrations of nicotine are present, receptor function may actually be a combination of both since different receptor subtypes also have very different sensitivities to both activation and desensitization. This phenomenon is readily demonstrated when comparing nAChRα7 receptors to nAChRα3/β4 receptors: nAChRα7 receptors are opened by relatively low nicotine concentrations but then rapidly desensitize, which is only very slowly reversible (possibly requiring complete receptor turn-over); nAChRα3/β4 receptors, which populate autonomic ganglia, are relatively insensitive to low concentrations of nicotine and while slow to desensitize, they rapidly reverse this state when nicotine amounts decrease. Therefore, a great number of the effects of nicotine on a system may reflect receptor desensitization rather than activation. Further, when assessing the role of nicotine in the system it becomes critical to distinguish if the exposure is acute or chronic, what concentration of nicotine is actually achieved in the system, and whether or not nAChR subtype expression would suggest activation, desensitization, or both.

Peripheral Sites of nAChR Expression and the Anti-inflammatory Effects of Nicotine

The importance of discriminating between the effects of nicotine versus the effects of tobacco becomes apparent when examining the literature contributing to the developing concept of the "cholinergic anti-inflammatory pathway," where nAChRs could prove to be valuable targets of therapeutic agents directed toward mediators of inflammation. Pavlov and colleagues[42] and Wang et al[43] first reported that acetylcholine or nicotine pretreatment of human peripheral blood mononuclear cells acted through a posttranscriptional mechanism to reduce the amount of tumor necrosis factor alpha (TNFα) present in the media 2 hours following stimulation with the bacterial component lipopolysaccharide (LPS). The anti-inflammatory properties of nicotine were specific in that other inflammatory cytokine production such as IL-1β, IL-18, and IL-6 were inhibited but not the anti-inflammatory cytokine IL-10. Under normal conditions (ie, in the absence of nicotine), it would appear that the vagus nerve release of acetylcholine at sites of peripheral tissue innervation provides the source of agonist for the nAChR. This idea is supported by the observation that vagotomy followed by LPS stimulation resulted in greater levels of TNFα in serum compared with control animals receiving LPS alone or sham vagotomy. The nAChR responsible for this effect was pharmacologically determined to be the nAChRα7 homomeric receptor,[43] which was later supported by studies in mice, where the nAChRα7 subunit was genetically eliminated. This group has also suggested[44] that nAChRα7 stimulation results in the decreased production of high mobility group box 1 (HMGB1) protein, which is a late mediator of lethal sepsis.

Of note, Pavlov[42] and Wang[43] were not the first to identify an interaction between nicotine (acting through nAChRα7) and TNFα. Numerous tissue culture studies have identified interactions between these pathways that impart neuroprotection to neurons challenged with excitotoxins such as NMDA.[7,45] An interesting aspect of these studies is that while either nicotine or acutely administered TNFα were neuroprotective, when applied together, neuroprotection was abolished.[7] This apparent antagonism between nAChRα7 and TNFα pathways has been explored further and apparently does not require the NFκB system or caspase activation.[10] Rather, the possibility exists that interactions are through modifications of the sphingomyelinase system as revealed through restoration of the neuroprotective effect by supplementation with ceramides.[46] Whatever the case, these studies collectively suggest an interaction between the signaling pathways initiated by the pro-inflammatory cytokine TNFα and nicotine with the nAChRα7 subtype.

Intestinal Epithelium

One of the earliest noted effects of nicotine on a peripheral tissue was in inflammation of the intestine. Early reports discussed patients with ulcerative colitis who upon cessation of smoking experienced more severe disease progression, which was ameliorated by returning to smoking.[17,20] In contrast, patients with Crohn's disease experienced severe disease when smoking, requiring the immediate and complete cessation of any tobacco product use.[18,19] Nicotine appears to be the key mediator of these responses as has been demonstrated by the use of transdermal patches, where their use inhibits inflammation associated with ulcerative colitis.[47,48] While nicotine has anti-inflammatory properties in this disease, the therapeutic value of nicotine does not exceed that of more conventional treatments such as aminosalicylates.[48] The mechanism through which nicotine acts in either of these diseases has not been resolved, and while both diseases are considered autoimmune in origin and thought to be related to the overproduction of inflammatory cytokines, their different etiologies and highly specific impact of nAChR expression or function is of great interest.

A fascinating issue to be addressed is which nAChRs, or possibly their respective expression levels, might participate in these disease states and the differential response to nicotine. A recent report by Orr-Urtreger et al[49] has taken on this difficult issue and found that mice deficient in the structural nAChR subunit α5 are more susceptible to experimentally induced inflammatory bowel disease than their wild-type controls. However, the story is complicated since transdermal nicotine attenuated the disease process to a certain extent in both wild-type and knockout mice, albeit more so in the knockout. This result suggests that the absence of nAChRα5 alters (increases) the susceptibility to disease initiation and the presence of nAChRα5 in the wild-type animal appears to enhance therapeutic sensitivity to nicotine. Of course, this again brings up many questions including whether nAChR composition or function may differ between Crohn's and ulcerative colitis patients. An additional note is that while a great deal of attention has been given to nAChRα7 in peripheral disease and inflammation, this exciting result suggests that it is premature to assume that this receptor is alone in its participation in modulating the peripheral inflammatory status. In fact, nAChR subunit mRNA for α3, α5, β2, and β4[50] has been detected in multiple cell types of the intestine suggesting that, as in the brain, nicotine may impact upon different inflammatory processes with considerable specificity depending upon the nAChR subtypes present. Such speculation is supported by another interesting report regarding the possible role of nAChR in disease processes of the gut where Richardson et al[51] found the absence of nAChRα3, normally expressed by ganglion cells, muscle, and epithelium of the small bowel, to be associated with a rare intestinal disease of childhood. Further, Xu et al[52] report that mice lacking nAChRα3 or both nAChRβ2 and nAChRβ4 have similar autonomic dysfunction of the bowel. Whether direct interaction with nAChRs expressed by epithelium or receptor expression by ganglia contributes to these disease processes remains to be determined.

Lung

Expression of nAChR subunits by epithelial and endothelial cells in the lung has also been observed.[15,53-57] This includes primary cultures of human bronchial cultured epithelial cells (BEC) that have been tested for nAChR expression. These cells express nAChRα7 mRNA and α-bungarotoxin binding that is indicative of mature receptor expression.[58] Other nAChR subunits present include nAChRα3, α5, β2, and β4 as well as the other potentially homomeric receptors composed of α9 and α10 that have been found using reverse transcriptase-polymerase chain reaction (RT-PCR) on human airway cells.[59] BEC also express acetylcholine, choline acetyltransferase, and the choline high affinity transporter, suggesting that acetylcholine may function as an autocrine or paracrine hormone for bronchial epithelial cells. Another source of agonist could be choline,[60] a compound that is transiently made available either in serum or locally following ingestion of fatty foods or during membrane remodeling. Choline is a full agonist of nAChRα7 and at high concentrations can exhibit activity toward other nAChRs.[60]

What are the implications of nAChR expression in the lungs with regard to both smokers and nonsmokers? While smoking is a major causative factor for lung cancer, relatively few smokers generate chronic obstructive pulmonary disease (COPD), which has an incidence rate of ~20%, even among very heavy and long-term smokers.[61] In fact, while the lungs of healthy smokers contain elevated numbers of activated macrophages, pulmonary Langerhans' cells, and primed neutrophils,[62] these cells actually exhibit reduced surface expression of major histocompatibility complex (MHC) class II molecules and costimulatory T lymphocyte molecules relative to controls.[63] Therefore, while cells are present that are primed for activation, the immune and inflammatory response may be dampened, as has been observed in many smokers.[62] Expanding upon this possibility, Floto and Smith[64] suggested that the inflammatory response to the stimulatory components of tobacco may be counteracted by the anti-inflammatory effects of nicotine, which offers a rational explanation for why few smokers generate pulmonary Langerhans' cell histiocytosis. It has also been pointed out that there is a significantly lower incidence of sarcoidosis in smokers[65] and decreased incidence of immunoglobulin G (IgG) precipitins that develop during allergic alveolitis as occurs both in humans and guinea pigs.[66,67] Whether this is due to the anti-inflammatory effects of nicotine is a direction of future research, especially with regard to the genetics regulating expression of nAChRs or the individual's genetic background. An encouraging feature of dissecting these complex traits and interactions is the use of mouse model systems. Mice have proven to be an extremely valuable model for translational studies related to basic issues of immunological function and show considerable promise in bringing similar experimental enlightenment to both genetic and environmental aspects of sensitivity to peripheral and central effects of nicotine including COPD. Differential susceptibility of mouse strains to nicotine have been extensively studied by the Collins group.[68] Further, the expression of nAChRs in the central nervous system can differ substantially in both cell types expressing these receptors and their organization in the brain,[69] in a mouse strain-dependent manner. These studies, as well as familial studies of addiction, point to the very evident genetic regulation of responsiveness to nicotine. Similarly, there is a mouse strain-dependent response to the development of emphysema in cigarette smoke-exposed mice.[70] Of the mouse strains tested, NZW/Lac/J, C57BL/6, A/J, SJL, and AKR (which all differ in MHC haplotypes), the AKR mice demonstrated an enhanced

susceptibility to the development of emphysema, while the C57BL/6, A/J, and SJL mice were mildly susceptible. The NZW/Lac/J mouse strain was resistant to emphysema and demonstrated a milder inflammation with reduced macrophage numbers and a remarkable lack of CD4+ or CD8+ T lymphocytes. Characterization of nAChR expression and function as they pertain to the genetics of these varied mouse strains would certainly enhance our understanding of these results.

Adipose Tissue

Smoking is a predisposing factor for insulin-resistance, which is associated with type 2 diabetes. Smokers are insulin-resistant and hyper-insulinaemic, and men who smoke are 4 times more likely to develop diabetes.[71] This is particularly interesting since type 2 diabetes is usually associated with obesity (excess adipose tissue) in adults; however, smokers are well established to be leaner, suggesting that simple explanations such as decreased eating is not the prime cause of this effect. In female smokers (but not necessarily in males), it is the fear of weight gain that often poses the greatest obstacle to smoking cessation.[72] Also of note is that exposure of mice to cigarette smoke for 6 months[70] resulted in weight loss in certain mouse strains (C57BL/6, AKR) but not others (A/J, SJL, NZW).

The role of the nAChRs in adipose tissue is only beginning to be explored as suggested by the limited and somewhat confusing literature. Miyazaki et al[73] have reported that smoking correlates significantly with decreased adiponectin levels in human plasma, which is usually associated with obesity and type 2 diabetes. Does a decrease in plasma adiponectin levels play a role in the generation of insulin-resistance in smokers even in the absence of obesity? Unfortunately, the function of adiponectin is not well understood. In a study to determine the role of nicotine on adipocytes, Liu et al[74] cultured rat adipocytes and found that mRNA for most of the neuronal and muscle nAChR were present in these cells, which were also found to bind [^{3}H]-nicotine. Further, pretreatment of these rat adipocytes with nicotine resulted in a reduced release of the pro-inflammatory cytokine TNFα as well as free fatty acids. Adiponectin levels in these adipocyte cultures, contrary to what might be expected from the in vivo studies, were elevated upon treatment with nicotine. Therefore, much remains to be explored in order to clarify the role of nAChR in the generation of smoke-induced insulin-resistance and metabolic syndrome with the occurrence of reduced adiposity.

Immune System

The relationship of nAChRs to immune function has 2 principal aspects. One resides in the realm of autoimmune attacks directed toward the expression of nAChRs, while the other addresses issues of nAChR expression by cells of the immune system and aspects of this relationship that are reflected in well-established observations such as the negative effect smoking has on the ability to fight infection. Autoimmune recognition of muscle nAChR at the neuromuscular junction resulting in myathenia gravis (MG)[75-77] continue to elucidate immune-mediated processes of this disease. While MG is the prototypical example of autoimmunity to the α1 subunit of the muscle nicotinic AChR, recent evidence suggests that other nAChR subunits[78] may also be targets in other autoimmune pathologies. One such subunit is α3, which when introduced into rabbits induced an autoimmune response with resulting autonomic neuropathy (AAN).[78] Further, this animal model replicates findings in some patients with idiopathic autonomic failure.[78] Such autoimmune recognition may also extend to nAChRα7, which contains amino acid sequences that are conditionally subject to proteolytic cleavage by granzyme B released from activated cytotoxic T cells.[79] Epitopes generated by cleavage of proteins with granzyme B tend to be autoantigenic.[80] Therefore, α7 cleavage by activated T cells is likely to be an excellent candidate as a target of presently unidentified autoimmune processes. While MG and AAN are peripheral pathologies, autoimmune processes against neuronal receptors may not be limited to peripheral diseases. For example, we have described[81] the presence and function of autoantibodies to a glutamate receptor (GluR3) in patients with Rasmussens encephalitis (RE), which is a rare pediatric seizure disorder characterized by intractable epilepsy. Many of these patients demonstrate anti-GluR3 antibodies that activate glutamate receptors on neurons. Further, GluR3 has a granzyme B site at the autoimmune epitope.[81] Lennon et al[82] have suggested that immune responses generated against nAChR may also result in seizure and dementia.

In the second arena of nAChR-immune cell interaction, chronic smoking affects both humoral and cell-mediated immune responses in rodents, monkeys, and humans.[62,83] Both thymic epithelium and thymocytes[84] express nAChR as do mature lymphocytes.[85,86] It has also been established that macrophages and dendritic cells also possess nAChR subunits. Exposure to cigarette smoke tends to suppress the response to infection.[87] For example, Kalra et al[88] demonstrated that smoking impairs antigen-mediated signaling in T cells and depletes IP3-sensitive Ca^{2+} stores, and van Dijk et al[89] have shown that transdermal nicotine (2 weeks on patch, normal volunteers) significantly reduced IL-2 production as well as a reduction in TNFα and IL-10 by blood cells stimulated with mitogen. A notable outcome of this effect may be that the anti-inflammatory properties of nicotine actually enhance the survival of influenza virus in mice and induce significantly higher titers of virus following

infection.[63] Pneumonia caused by *Streptococcus pneumonia*, is also more frequent in smokers.[90]

Macrophages are critical effector cells for early recognition and destruction of organisms invading through most surfaces including the gut, skin, and lung. Matsunaga el al[91] have shown that nicotine treatment of a mouse alveolar macrophage cell line (expressing α4 and β2, but not α7) results in enhanced intracellular replication of *Legionella pneumophilia*. Further, the production of the inflammatory cytokines IL-6, TNFα, and IL-12 were down-regulated in these cells. While nicotine may inhibit macrophage function to promote pneumonia, it has also been reported that the generation of hypersensitivity pneumonia (HP) is lower in smokers than nonsmokers. HP is caused by inhalation of antigens such as *Saccharopolyspora rectivirgula,* which induces farmer's lung (once contracted, however, smoking worsens disease). Blanchet et al[92] observed that nicotine-induced inhibition of macrophage function may actually protect against inflammatory lung processes such as HP by decreasing the number of alveolar macrophages in the lungs of experimental animals and decreasing inflammatory cytokine production. Further, Shivji et al[93] reported recently that chronic nicotine exposure results in a reduction of lung S-adenosylmethionine (AdoMet), which is required for growth of *Pneumocystitis carinii*. As such, smoking and nicotine protect against *Pneumocystitis*. A clinical correlate of protection in humans against *Pneumocystitis*, which is closely associated with AIDS infection, has been reported by Saah et al.[94]

While macrophages initiate many inflammatory and innate immune functions, dendritic cells (DCs) are the principal antigen-presenting cells. Aicher et al[95] demonstrated that low doses of nicotine induced the expression of molecules with costimulatory activity toward antigen presentation and increased the secretion of IL-12 (pro-inflammatory) by TH1 T lymphocytes by 7-fold. This effect was mediated by PI3-kinase, AKT, and p38 MAPK. The overall effect observed was an increase in dendritic-cell stimulation of T-cell proliferation and cytokine secretion. They also report that DCs are increased in atherosclerotic plaques and that this effect of nicotine may contribute to progression of atherosclerotic lesions.

Skin

The relationship between smoking and skin has been a topic of investigation for a long period. This is in part because studies have suggested that smoking is a risk factor in development of premature facial wrinkling.[96,97] While not all studies necessarily agree with smoking contributing to increased wrinkling, they do conclude that smokers on average look older and suggest that smoking increases aging of the skin. Mechanistically, nicotine can impact upon epithelial keratinocytes (KC) that express nAChR receptor subunits as well as the enzymes for acetylcholine synthesis, which may act as cell signalling molecules ("cytotransmitters") as has been suggested for the lung.[53] Grando and colleagues[98] established that KCs express nAChRs and that these receptors respond functionally to nicotine. Kurzen et al[99] localized nAChR subunit expression in the skin and conclude that there is a very distinctive pattern of subunit expression in normal human epidermis. Perhaps more important than issues pertaining to premature wrinkling is the relationship between smoking (and nicotine use) and delayed wound healing. For example, nicotine inhibits keratinocyte migration[100,101] slowing wound healing. nAChRα7 expression by KC is implicated in underlying this observation, where it appears to play an important role in cell cycle progression, apoptosis, and differentiation.[102] This is particularly supported by studies of α7 knockout mice,[103] where the expression of pro-apoptotic proteins Bad and Bax are reduced, and an antagonist of muscarinic and nAChRα4/β4[104] cholinergic receptors (atropine) decreases the expression of desmoligein (Dsg), which is an important regulator of cell adhesion. How these results translate into biologic effects of nicotine on skin remains to be determined; however, the possibility exists that, through inhibition of apoptosis, tumorgenesis is also enhanced. Therefore, the reports of nicotine effects on the skin appear rather deleterious, although separating nicotine effects from those of cigarette smoke for skin effects has not been extensively examined in vivo. Future analysis of epidemiological data from subjects using nicotine delivery through the skin-patch will be of particular value in assessing these effects.

Oral Epithelium

The major preventable risk factor for periodontal disease is smoking, and there is a direct correlation between cigarette number and risk. Exposure to second-hand smoke has also been suggested to enhance periodontal risk by up to 20-fold,[105] and both smoking and nicotine have been reported to increase inflammation by reducing oxygen in gum tissue and initiating over-production of the inflammatory cytokines, which in excess are harmful to cells and tissue. Furthermore, when nicotine combines with oral bacteria, such as *Porphyromonas gingivalis*, the effect produces even greater levels of cytokines and eventually leads to periodontal connective-tissue breakdown.[106] Studies suggest that smokers are 11 times more likely than nonsmokers to harbor the bacteria that cause periodontal disease and 4 times more likely to have advanced periodontal disease. In one study, more than 40% of smokers lost their teeth by the end of their lives. Oral epithelial cells express nicotinic receptors. Arredondo et al[107] reported that both nicotine and environmental tobacco smoke (ETS) increased expression of regulators of the cell cycle and apoptosis. This up-regulation

was inhibited by transfection into human keratinocytes from gingival of small interfering RNA for human nAChRα3. Further, the α3 knockout mouse did not generate these changes in gene expression with exposure to ETS or nicotine. These investigators[107] also report the presence of α5, α2, and the muscarinic receptors M2 and M3.

Endothelium

Smoking is a major risk factor for cardiovascular disease, and nicotine plays a role in some of the pathogenic processes involved. For example, Heeschen et al[108] have demonstrated that nicotine increases endothelial cell number, reduces apoptosis, increases capillary network formation, and accelerates the growth of atherosclerotic plaques. Growth of plaques is dependent on vascularization, which is increased in nicotine-treated mice. Macklin et al[54] find that human aortic endothelial cells that line blood vessels express functional nAChRs with α3 being a predominant subunit. These endothelial nAChR demonstrate similar iongating properties as those on ganglionic neurons. Further, these investigators have shown that acetylcholine is also produced by endothelial cells, suggesting that an autocrine mechanism of activation can occur. An intriguing discussion by these authors suggests that the effect of nicotine on desensitization (see above) of these receptors may make endothelial cells nonresponsive to the endogenously produced acetylcholine and that this nonresponsiveness should be considered in the pathogenesis of atherosclerosis. Intracellular mechanisms that are activated by nicotine in endothelial cells have been demonstrated by Di Luozzo et al[109] and include MAPKs p38 and p44/p42. The receptors present on rat endothelial cells, as revealed[110] by RT-PCR include nAChRs α2, α3, α4, α5, α7, β2, and β4 but not β3. Saeed et al[111] have demonstrated that nicotine inhibits the expression of endothelial adhesion molecules, suggesting that the effect of nicotine on these cells is anti-inflammatory. Therefore, both pro- and anti-inflammatory mechanisms may be involved in nicotine-mediated effects on endothelium and again points to the need for determination of the nAChR receptor subunits involved.

CONCLUSION

A frequently reported influence of nAChR expression and function by tissues of the periphery is on inflammation. Because the inflammatory response is an integral and specialized process in all tissues, this suggests that an equally broad and specific range of nAChR-related outcomes can be expected that may be both beneficial and harmful to the host. For example, controlling the inflammatory response in ulcerative colitis would ameliorate tissue damage. In contrast, suppressing the inflammatory response during an innate (or adaptive) immune response through chronic nAChR activation/desensitization would dampen this reaction and impact in a negative way on the rate of clearing of a microbial infection or the efficacy of long-term protection against recurrent challenges by foreign substances. In addition to the direct influence of chronically activated/desensitized nicotinic receptors, it is also important to consider that the many inflammatory processes as well as responses to nicotine are highly predisposed to genetic background as witnessed by the broad diversity of the influence of nicotine on mice of differing strain background. The effective usefulness of nAChR-based strategies will ultimately depend upon a clear understanding of the collective biological consequences of peripheral nAChR expression on inflammation, and it should also be considered that they will have the possibility of meeting with undesirable side-effects. For example, the anti-inflammatory properties of nicotine that promote neuronal survival in the aging brain could also encourage survival of proliferating cells in the periphery that should otherwise die, produce immune suppression, or even influence metabolism and insulin resistance. However, coupled to this complexity is the exciting prospect that a detailed understanding of how nAChRs impart these diverse effects will be rewarded with many novel therapeutic strategies.

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